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# Bioelectrochemical Fixation of Carbon Dioxide with Electric Energy Generated by Solar Cell

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## 1. Introduction

Atmospheric carbon dioxide has been increased and was reached approximately to 390 mg/L at December 2010 (Tans, 2011). Rising trend of carbon dioxide in past and present time may be an indicator capable of estimating the concentration of atmospheric carbon dioxide in the future. Cause for increase of atmospheric carbon dioxide was already investigated and became general knowledge for the civilized peoples who are watching TV, listening to radio, and reading newspapers. Anybody of the civilized peoples can anticipate that the atmospheric carbon dioxide is increased continuously until unknowable time in the future but not in the near future. Carbon dioxide is believed to be a major factor affecting global climate variation because increase of atmospheric carbon dioxide is proportional to variation trend of global average temperature (Cox et al., 2000). Atmospheric carbon dioxide is generated naturally from the eruption of volcano (Gerlach et al., 2002; Williams et al., 1992), decay of organic matters, respiration of animals, and cellular respiration of microorganisms (Raich and Schlesinger, 2002; Van Veen et al., 1991); meanwhile, artificially from combustion of fossil fuels, combustion of organic matters, and cement making-process (Worrell et al., 2001). Theoretically, the natural atmospheric carbon dioxide generated biologically from the decay of organic matter and the respirations of organisms has to be fixed biologically by land plants, aquatic plants, and photosynthetic microorganisms, by which cycle of atmospheric carbon dioxide may be nearly balanced (Grulke et al., 1990). All of the human-emitted carbon dioxide except the naturally balanced one may be incorporated newly into the pool of atmospheric greenhouse gases that are methane, water vapor, fluorocarbons, nitrous oxide, and carbon dioxide (Lashof and Ahuja, 1990). The airborne fraction of carbon dioxide that is the ratio of the increase in atmospheric carbon dioxide to the emitted carbon dioxide variation was typically about 45% over 5 years period (Keeling et al., 1995). Canadell et al. (2007) reported that about 57% of human-emitted carbon dioxide was removed by the biosphere and oceans. These reports indicate that the airborne fraction of carbon dioxide is at least 43-45%, which may be the balance emitted by human activity.

The land plants are the largest natural carbon dioxide sinker, which have been decreased globally by deforestation (Cramer et al., 2004). Especially, tropical and rainforests are being

cut down for different purpose and by different reason and some of the forest are being burned for slash and burn farming. The atmospheric carbon dioxide and other greenhouse gases are increased in proportion to the deforestation (McKane et al. 1995). Deforestation causes part of the released carbon dioxide to be accumulated in the atmosphere and the global carbon cycle to be changed (Robertson and Tiejai, 1988). The releasing carbon dioxide and changing carbon cycle increase the greenhouse effect and may raise global temperature. The greenhouse effect is generated naturally by the infrared radiation, which is generated from incoming solar radiation, absorbed into atmospheric greenhouse gases and re-radiated in all direction (Held and Soden). The gases contributing to the greenhouse effect on Earth are water vapor (36-70%), carbon dioxide (9-26%), methane (4-9%), and ozone (3-7%) (Kiehl et al., 1977). Especially, water vapor can amplify the warming effect of other greenhouse gases, such that the warming brought about by increased carbon dioxide allows more water vapor to enter the atmosphere (Hansen, 2008). The greenhouse effect can be strengthened by human activity and enhanced by the synergetic effect of water vapor and carbon dioxide because the elevated carbon dioxide levels contribute to additional absorption and emission of thermal infrared in the atmosphere (Shine et al., 1999). The major non-gas contributor to the Earth's greenhouse effect, cloud (water vapor), also absorb and emit infrared radiation and thus have an effect on net warming of the atmosphere (Kiehl et al., 1997). Elevation of carbon dioxide is a cause for greenhouse effect, by which abnormal climate, desertification, and extinction of animals and plants may be induced (Stork, 1997). However, carbon dioxide is difficult to be controlled in the industry-based society that depends completely upon fossil fuel. If the elevation of carbon dioxide was unstoppable or necessary evil, the technique to convert biologically the atmospheric carbon dioxide to stable polymer in the condition without using fossil fuel must be developed. All of the land and aquatic plants convert mainly carbon dioxide to biomolecule in coupling with oxygen generation; however, a total of 16.5% of the forest (230,000 square miles) was affected by deforestation due to the increase of fragmented forests, cleared forests, and boundary areas between the fragmented forests (Skole et al., 1998). Decline of plants may be a cause to activate generation of the radiant heat because the visible radiation of solar energy absorbed for photosynthesis can be converted to additional radiant heat.

Solar cell is the useful equipment capable of physically absorbing solar radiation and converting the solar energy to electric energy (O'Regan and Grätzel, 1991). The radiant heat generated from the solar energy may be decreased in proportion to the electric energy produced by the solar cells. Electrochemical redox reaction can be generated from electric energy by using a specially designed bioreactor equipped with the anode and cathode separated with membrane, which is an electrochemical bioreactor. The electric energy generated from the solar energy can be converted to biochemical reducing power through the electrochemical redox mediator. The biochemical reducing power (NADH or NADPH) is the driving force to generate biochemical energy, ATP. The biochemical reducing power and ATP are essential elements that activate all biochemical reactions for biosynthesis of cell structure and production of metabolites.

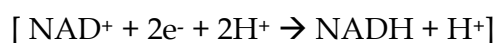
## 2. Electrochemical redox mediator

The electrochemical reduction reaction generated in cathode can't catalyze reduction of  $\text{NAD}^+$  or  $\text{NADP}^+$  both *in vitro* and *in vivo* without electron mediator. Various ion radicals that are methyl viologen, benzyl viologen, hydroquinone, tetracyanoquinodimethane, and

neutral red (NR) have been used as electron mediator to induce electrochemical redox reaction between electrode and electron carriers that are  $\text{NAD}^+$ , FAD, and cytochrome C (Pollack et al., 1996; Park et al., 1997; Wang and Du, 2002; Kang et al., 2007). In order to *in vivo* drive and maintain bacterial metabolism with electrochemical reducing power as a sole energy source, only  $\text{NAD}^+$  or  $\text{NADP}^+$  is required to be reduced by coupling redox reaction between electron mediator and biochemical electron carrier (Park and Zeikus, 1999; 2000). NR can catalyze the electrochemical reduction reaction of  $\text{NAD}^+$  both *in vivo* and *in vitro* but no electron mediator except the NR can. NR is a water-soluble structure composed of phenazine ring with amine, dimethyl amine, methyl, and hydrogen group as shown in Fig 1. The dimethyl amine group is redox center for electron-accepting and donating in coupling with phenazine ring; meanwhile, the amine, methyl, and hydrogen are structural group. Redox potential of NR is -0.325 volt (vs. NHE), which is 0.05 volt lower than  $\text{NAD}^+$ . The electrochemical redox reaction of NR can be coupled to biochemical redox reaction as follows:



$\text{NAD}^+$  can be reduced in coupling with biochemical redox reaction as follows:



Commonly,  $\text{NR}_{\text{ox}}$  and  $\text{NAD}^+$  are reduced to  $\text{NR}_{\text{red}}$  and  $\text{NADH}$ , respectively by accepting two electrons and one proton.

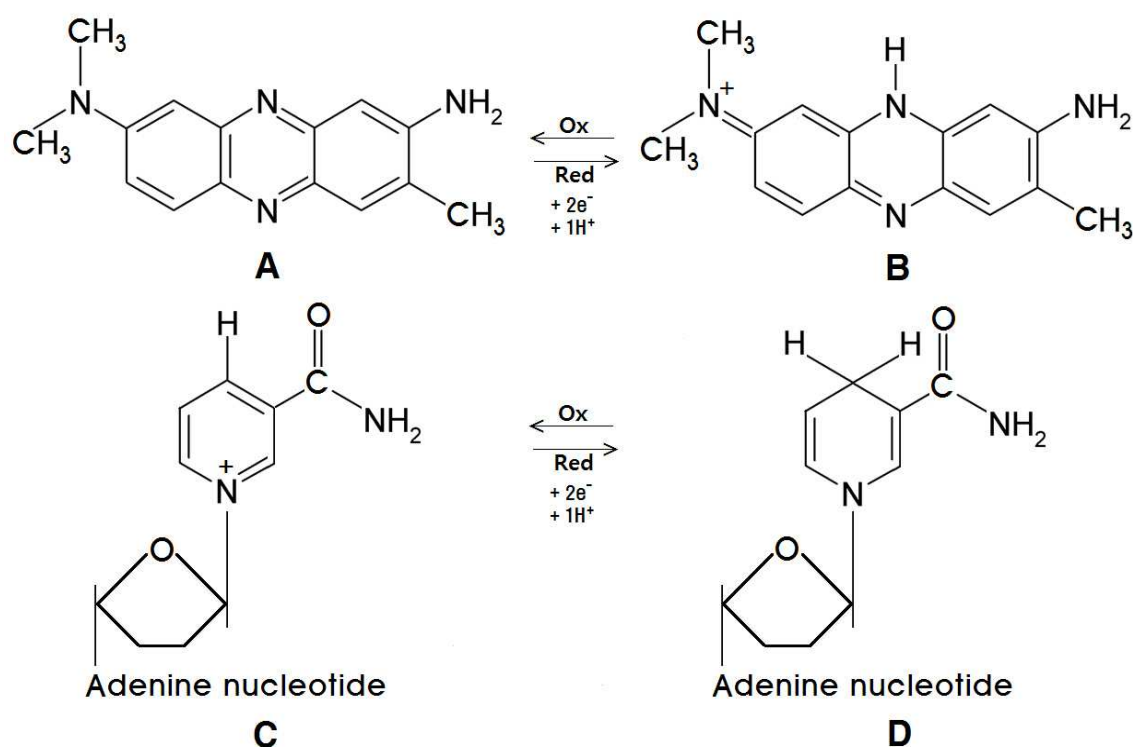


Fig. 1. Molecular structure of neutral red, which can be electrochemically oxidized (A) or reduced (B). The reduced neutral red can catalyze reduction reaction of  $\text{NAD}^+$  (C) to  $\text{NADH}$  (D) without enzyme catalysis. Ox and Red indicate oxidation and reduction, respectively.

Theoretically, the water-soluble NR may be reduced at the moment when contacted with electrode and catalyze biochemical reduction of  $\text{NAD}^+$  at the moment when contacted with bacterial cell or enzyme. A part of NR may be contacted with electrode or bacterial cell in water-based reactant but most of that is dissolved or dispersed in the reactant. In order to induce the effective electrochemical and biochemical reaction in the bacterial culture, NR and bacterial cells have to contact continuously and simultaneously with electrode. This can be accomplished by immobilization of NR in graphite felt electrode based on the data that most of bacterial cells tend to build biofilm spontaneously on surface of solid material and the graphite felt is matrix composed of  $0.47\text{m}^2/\text{g}$  of fiber (Park et al., 1999). The amino group of NR can bind covalently to alcohol group of polyvinyl alcohol by dehydration reaction, in which polyvinyl-3-imino-7-dimethylamino-2-methylphenazine (polyvinyl-NR) is produced as shown in Fig 2. The polyvinyl-NR is a water-insoluble solid electron mediator to catalyze electrochemically reduction reaction of  $\text{NAD}^+$  like the water-soluble NR (Park and Zeikus, 2003). The polyvinyl-NR immobilized in graphite felt (NR-graphite) functions as a cathode for electron-driving circuit, an electron mediator for conversion of electric energy to electrochemical reducing power, and a catalyst for reduction of  $\text{NAD}^+$  to NADH. The electrochemical bioreactor equipped with the NR-graphite cathode is very useful to cultivate autotrophic bacteria that grow with carbon dioxide as a sole carbon source and electrochemical reducing power as a sole energy source (Lee and Park, 2009).

### 3. Separation of electrochemical redox reaction

The biochemical reducing power can be regenerated electrochemically by NR-graphite cathode (working electrode) that functions as a catalyst, for which  $\text{H}_2\text{O}$  has to be electrolyzed on the surface of anode (counter electrode) that functions as an electron donor. The working electrode is required to be separated electrochemically from the counter electrode by specific septa that are the ion-selective Nafion membrane (Park and Zeikus, 2003; Kang et al., 2007; Tran et al., 2009), the ceramic membrane (Park and Zeikus, 2003; Kang et al., 2007; Tran et al., 2009), the modified ceramic membrane with cellulose acetate film (Jeon et al., 2009B), and the micro-pored glass filter, by which the electrochemical reducing power in the cathode compartment can be maintained effectively. Jeon and Park (2010) developed a combined anode that was composed of cellulose acetate film, porous ceramic membrane and porous carbon plate as shown in Fig 3. The combined anode functions as a septum for electrochemical redox separation between anode and cathode, an anode for electron-driving circuit, and a catalyst for electrolysis of  $\text{H}_2\text{O}$ . The major function of anode is to supply electrons required for generation of electrochemical reducing power in the working electrode (NR-graphite cathode), in which  $\text{H}_2\text{O}$  functions as an electron donor. The strict anaerobic bacteria that are methanogens, sulfidogens, and anaerobic fermenters grow in the condition with lower oxidation-reduction potential than  $-300\text{ mV}$  (vs. NHE) (Ferry, 1993; Gottschalk, 1985), which can be induced electrochemically inside of the carbon fibre matrices of NR-cathode under only non-oxygen atmosphere. The NR-cathode can catalyze biochemical regeneration of NADH and generation of hydrogen but can't catalyze scavenging of oxygen and oxygen radicals at around  $25^\circ\text{C}$  and  $1\text{ atm}$ . The combined anode can protect effectively contamination of catholyte with the atmospheric oxygen by unidirectional evaporation of water from catholyte to atmosphere through the combined anode as shown in Fig 4. The driving force for the unidirectional evaporation of water may be generated naturally by the difference of water pressure between catholyte and outside atmosphere (Jeon et al., 2009A).

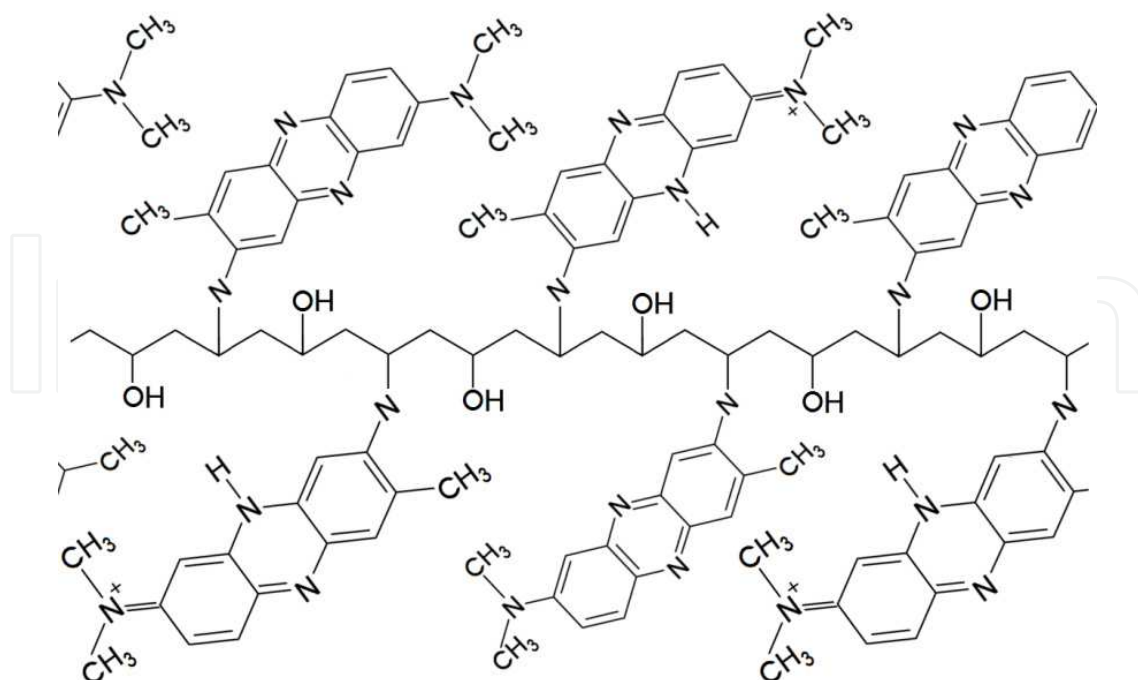


Fig. 2. Schematic structure of polyvinyl-NR that is produced by covalent bond between amine of NR and alcohol of polyvinyl alcohol. The polyvinyl-NR can bind physically to graphite cathode surface.

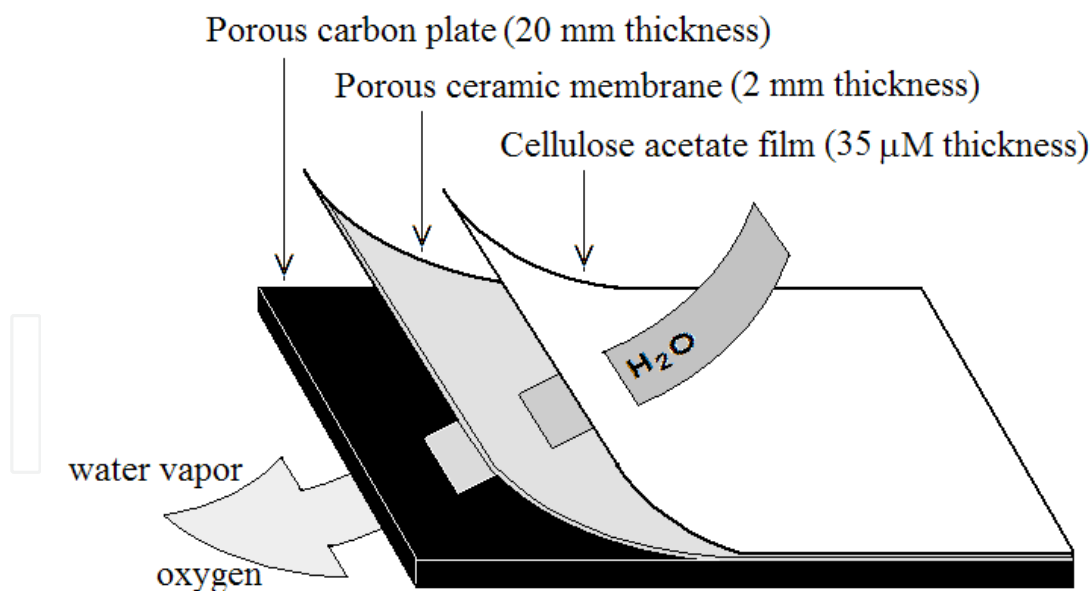


Fig. 3. Schematic structure of a combined anode composed of cellulose acetate film, porous ceramic membrane and porous carbon plate. Water or gas can penetrate across the cellulose acetate film but solutes can't.

Practically, the hydrogenotrophic methanogens are useful microorganisms for carbon dioxide fixation using the electrochemical bioreactor. However, most of the reducing power that is electrochemically generated in the NR-graphite cathode may be consumed to

maintain the proper oxidation-reduction potential for growth of the hydrogenotrophic methanogens in the condition without chemical reducing agent. This may be a cause to decrease the regeneration effect of the biochemical reducing power and free energy in the electrochemical bioreactors. In natural ecosystem, hydrogen sulfide produced metabolically by sulfidogens in coupling with oxidation of organic acids functions as the chemical reducing agent to maintain the proper environmental condition for growth of the methanogens (Thauer et al., 1977; Oremland et al., 1989; Zinder et al., 1984).

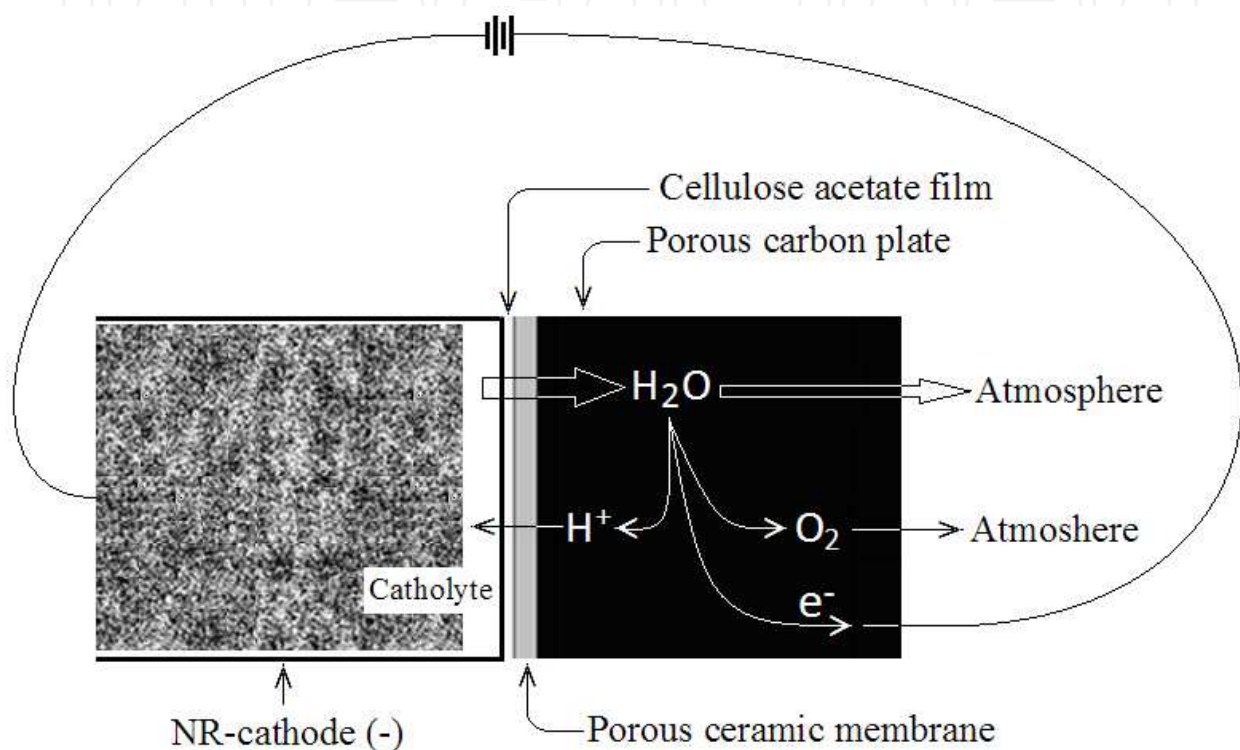


Fig. 4. Schematic structure of the combined anode composed of cellulose acetate film, porous ceramic membrane, and porous carbon plate, in which protons, electrons, and oxygen generated from water by the electrolysis may be transferred separately to the catholyte, the NR-cathode, and the atmosphere. Water is transferred from catholyte to atmosphere through the combined anode by difference of water pressure between catholyte and atmosphere.

Meanwhile, the growth condition for facultative anaerobic mixotrophs is not required to be controlled electrochemically because the metabolic function of the facultative anaerobic mixotrophs is not influenced critically by the oxidation-reduction potential. Accordingly, the combined anode may be replaced by the glass filter (pore, 1-1.6  $\mu\text{m}$ ) that permits transfer of water and diffusion of ions and soluble compounds. Water transferred from catholyte to anolyte through the glass filter by difference of pressure and volume is electrolysed into oxygen, protons, and electrons in the anode compartment. The protons, electrons, and oxygen are transferred separately to the catholyte, the NR-cathode, and the atmosphere as shown in Fig 5. The water in the anode compartment equipped at the center of catholyte is consumed continuously by electrolysis and refilled spontaneously from catholyte by difference of volume and pressure between the catholyte and anolyte.

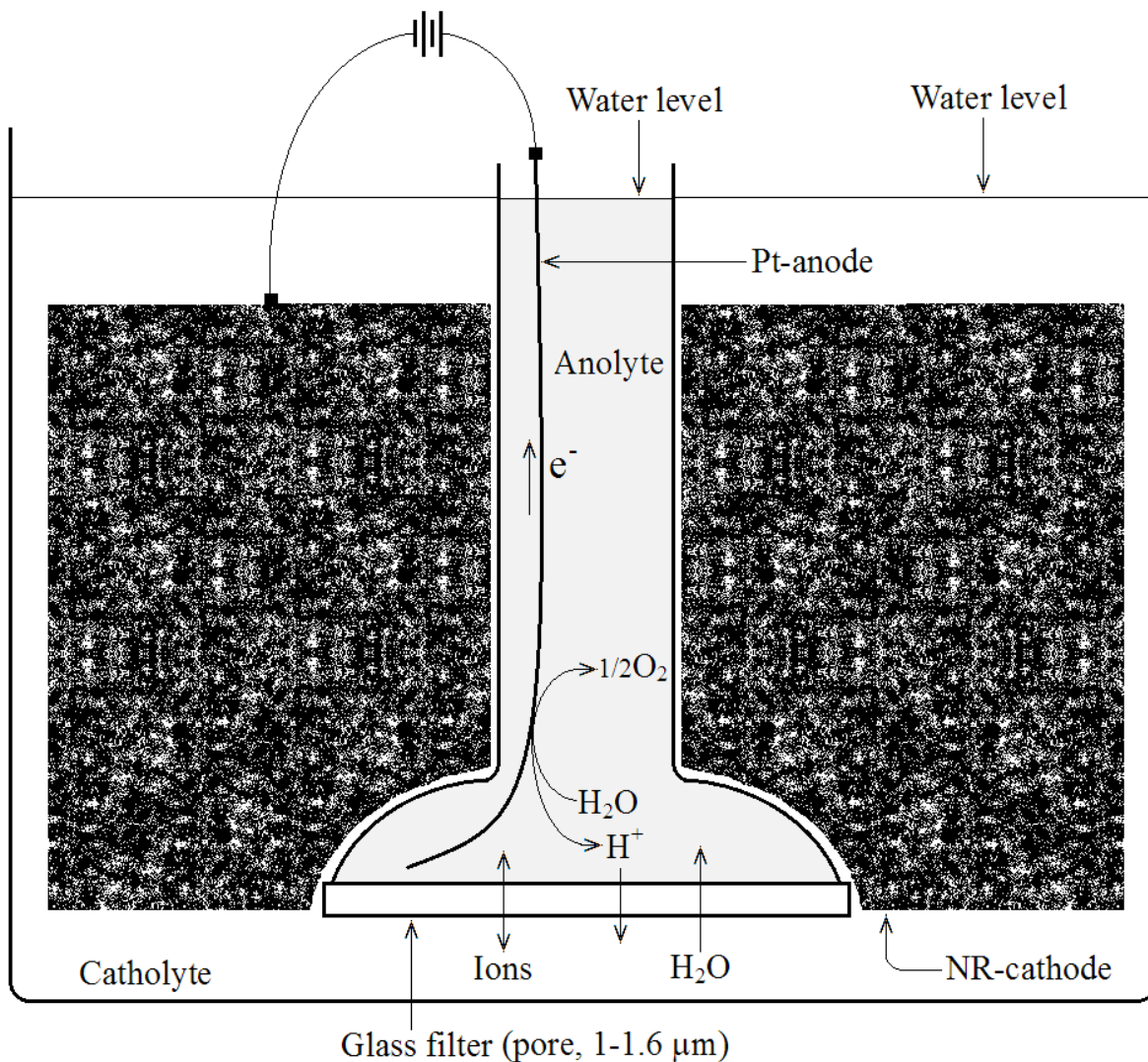


Fig. 5. Schematic structure of the anode and cathode compartment separated by glass filter. Protons, electrons, and oxygen generated from water by the electrolysis may be transferred separately to the catholyte, the NR-cathode, and the atmosphere.

#### 4. Enrichment of hydrogenotrophic methanogens

A specially designed electrochemical bioreactor is composed of the combined anode (Fig 4) and NR-graphite cathode for enrichment of the hydrogenotrophic methanogens as shown in Fig 6. Oxygen-free and carbonate-saturated wastewater was supplied continuously from a wastewater reservoir as shown in Fig 7. The electrochemical bioreactor was operated with the electricity generated from the solar panel. The wastewater obtained from sewage treatment plant was used without sterilization, to which 50 mM of sodium bicarbonate was added. The contaminated oxygen was consumed by bacteria growing intrinsically in the wastewater reservoir. Hydrogenotrophic methanogens grow with the free energy and reducing power generated by the coupling redox reaction of carbon dioxide and hydrogen (Ferguson and Mah, 1983; Na et al., 2007; Zeikus and Wolfe, 1972). Hydrogen generated from the electrolysis of water can't function to maintain the proper oxidation reduction



potential for methanogenic bacteria in the electrochemical bioreactor owing to the micro-solubility. The micro-pore formed by the fiber matrices of NR-graphite cathode may be proper micro-environment for the growth of hydrogenotrophic methanogens because hydrogen generated from NR-graphite cathode may be captured in the micro-pores and the lower oxidation-reduction potential than  $-300$  mV (vs. NHE) may be maintained by the electrochemical reducing power.

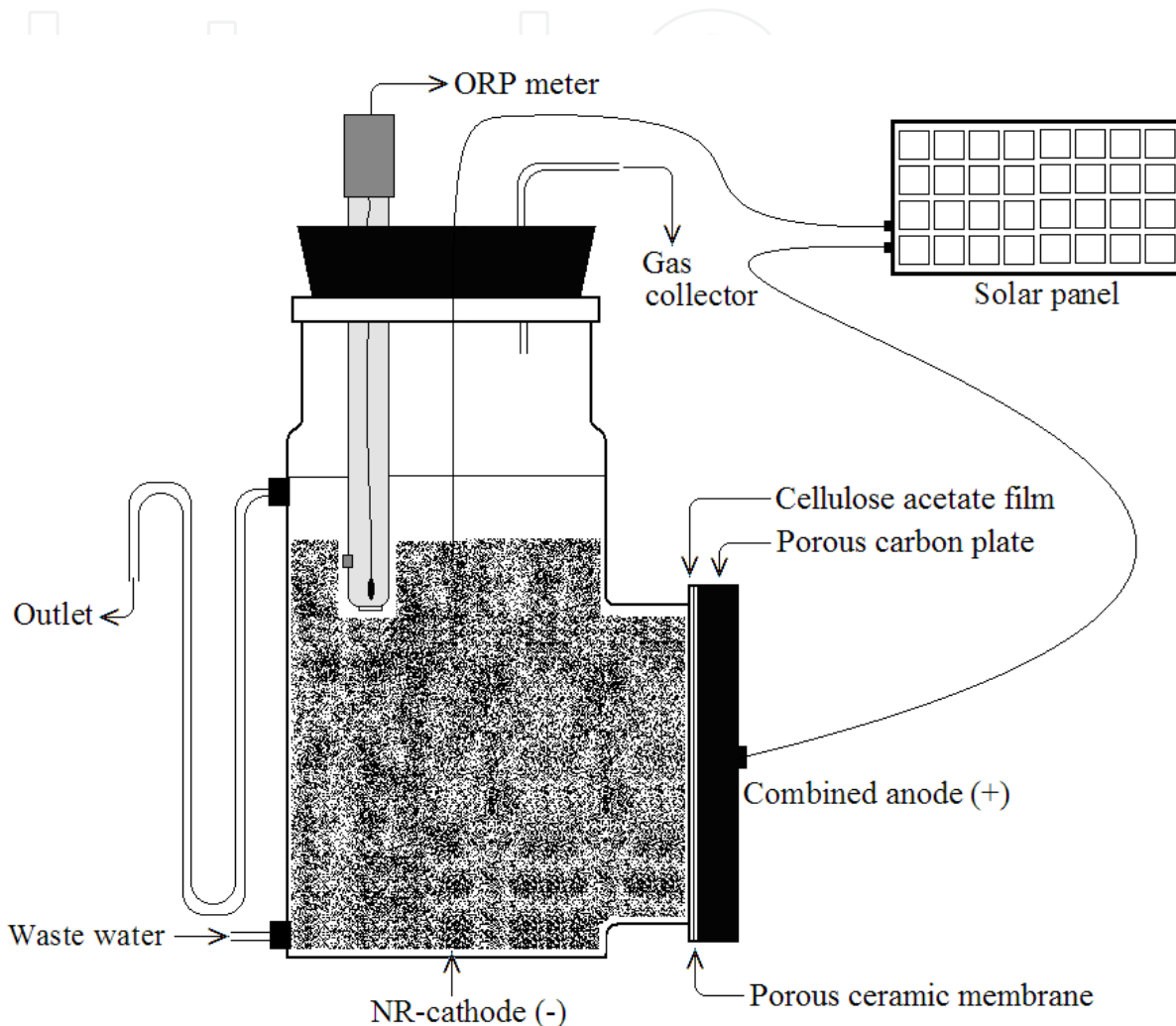


Fig. 6. Schematic structure of an electrochemical bioreactor, in which the anode compartment was replaced with the combined anode composed of cellulose acetate film, porous ceramic membrane and porous carbon plate. Water is electrolyzed in the porous carbon plate and separated into proton, electron, and oxygen.

Methyl compounds, hydrogen, low molecular weight fatty acids, hydrogen, and carbon dioxide are produced by various fermentation bacteria in the anaerobic digestive sludge. The methanogens grow syntrophically in the bioreactor cultivating anaerobic digestive sludge, which is composed of various organic compounds and anaerobic bacterial community (Stams et al., 2009; Katsuyam et al., 2009). When the anaerobic digestive sludge was applied to the electrochemical bioreactor (Fig 6 and 7), the hydrogenotrophic methanogens that are *Methanobacterium* sp., *Methanolinea* sp., and *Methnoculleus* sp. were enriched predominantly (Jeon et al., 2009B). The predominated hydrogenotrophic methanogens consumed and

produce actively carbon dioxide and methane, respectively, using the electrochemical reducing power generated from the solar panel (Cheng et al., 2011). Practically, the methane production and carbon dioxide consumption were significantly increased in the electrochemical bioreactor as shown in Fig 8.

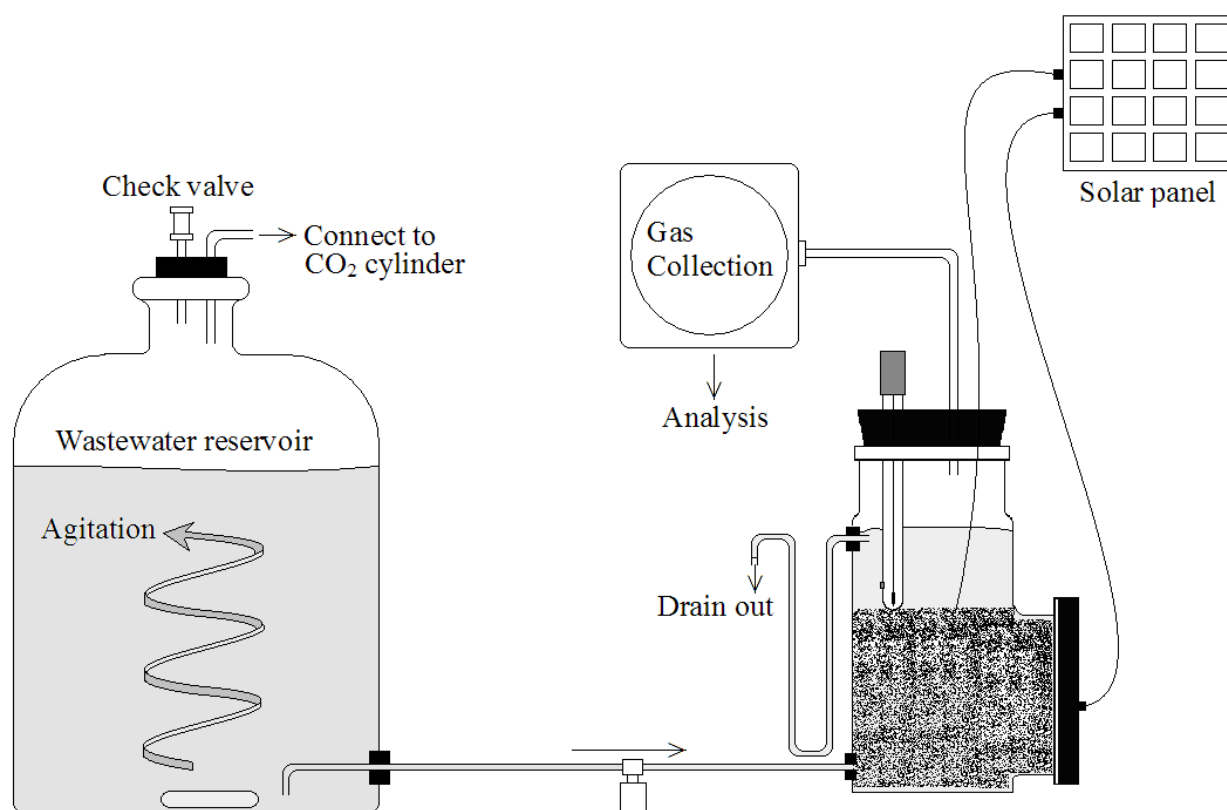


Fig. 7. Schematic structure of an electrochemical bioreactor for continuous culture of hydrogenotrophic methanogens. The wastewater saturated with carbon dioxide is supplied continuously to the electrochemical bioreactor and headspace of wastewater reservoir was refilled continuously with pure carbon dioxide without oxygen contamination.

Bacteriological conversion of carbon dioxide to methane using the electrochemical reducing power may be a technique for fixation of carbon dioxide without combustion of fossil fuel; however, may not be a way for long term storage of carbon. Cell structures of bacteria are composed of peptidoglycan, phospholipid, proteins, nucleic acids, and carbohydrates that are biochemically stable polymers (Caldwell, 1995). Bacterial cells themselves can be the carbon storage by freezing or drying without the specific engineering process. Hydrogenotrophic methanogens may not be proper carbon storage because they consume the reducing power and free energy ineffectively to maintain the lower oxidation-reduction potential than -300 mV, grow more slowly than other autotrophic bacteria, and produce the unstable metabolite (methane).

Facultative anaerobic mixotrophs, on the other hand, not only grow heterotrophically in the condition with organic carbons but also grow autotrophically in the condition with electron donors and carbon dioxide (Johnson, 1998; Morikawa and Imanaka, 1993). The metabolism and physiological function of the facultative anaerobic mixotrophs are not influenced by oxygen. These are useful character of the facultative anaerobic mixotrophs to cultivate with

wastewater containing other reduced organic and inorganic compounds and exhaust containing carbon dioxide, and the electrochemical reducing power as the electron donor (Skirnisdottir et al., 2001).

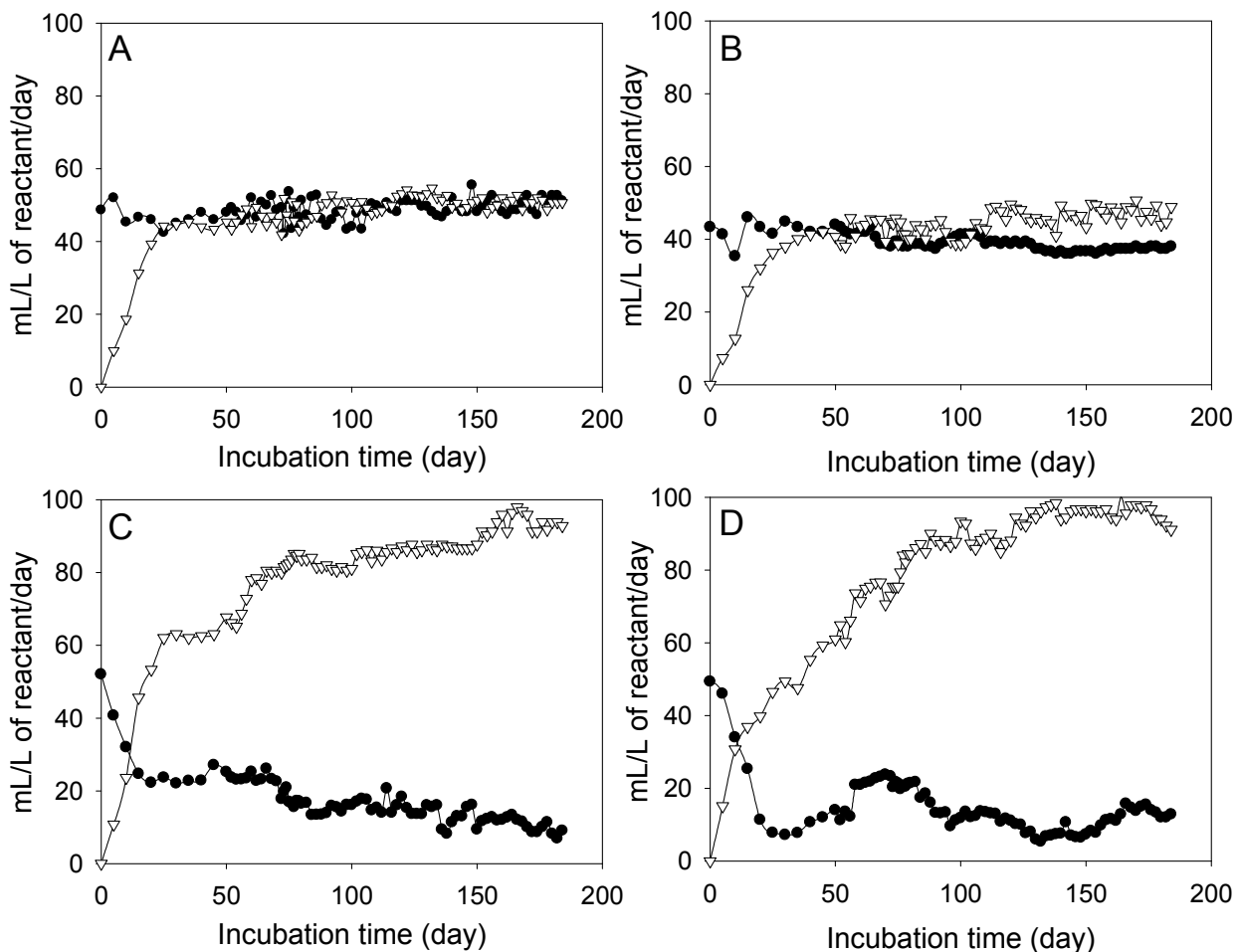


Fig. 8. Carbon dioxide consumption (●) and methane production (▽) by anaerobic digestive sludge cultivated in conventional bioreactor (reactors A and B) and electrochemical bioreactor (reactors C and D). Duplicate reactors were operated to enhance the comparability between the conventional bioreactor and the electrochemical bioreactor.

### 5. Enrichment and cultivation of carbon dioxide-fixing bacteria

A cylinder-type electrochemical bioreactor composed of the built-in anode compartment and NR-graphite cathode was employed to enrich the facultative anaerobic mixotrophs capable of fixing carbon dioxide with electrochemical reducing power as shown in Fig 9. The NR-cathode was separated electrochemically from anode compartment by the glass filter (Fig 5). Mixture of the bacterial community obtained from aerobic wastewater treatment reactor, forest soil, and anaerobic wastewater was cultivated in the cylinder-type electrochemical bioreactor to enrich selectively carbon dioxide-fixing bacteria with the electrochemical reducing power generated from NR-graphite cathode. DC -3 volt of electricity that was generated by a solar panel was charged to NR-graphite cathode to induce generation of electrochemical reducing power. Electricity is the easiest energy to

transfer and supply to any electronic device. The electrochemical bioreactor is also the simplest electronic device to convert electric energy to biochemical reducing power. The wastewater and exhausted gas can be used directly without purification or separation as the nutrient source for bacterial metabolism. Experimentally, the electricity generated from the 25 cm<sup>2</sup> of the solar panel is very enough for operation of the 10 L of electrochemical bioreactor.

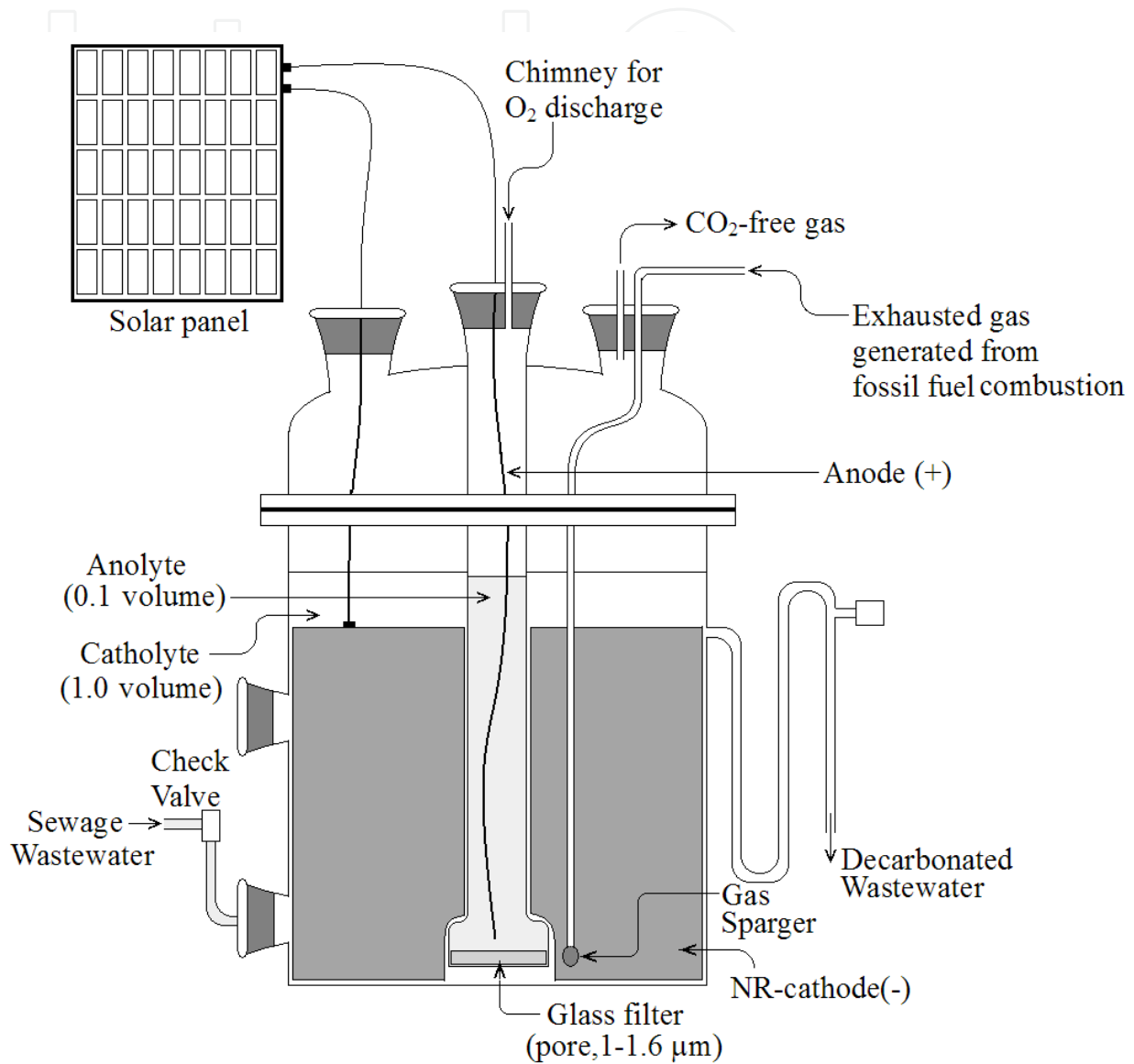


Fig. 9. Schematic diagram of the cylinder-type electrochemical bioreactor equipped with a built-in anode compartment for the cultivation of CO<sub>2</sub>-fixing bacteria. The glass filter septum equipped at the bottom end of the anode compartment functions as redox separator between anode and cathode compartment and micropore for transfer of catholyte to anode compartment.

During enrichment of the carbon dioxide-fixing bacteria using the cylinder-type electrochemical bioreactor, bacterial community was changed significantly as shown in Fig 10. Some of bacteria community was increased or enriched as shown in the box A and C but decreased or died out as shown in the box B and D. These phenomena are a clue that the

bacterial species that can fix carbon dioxide with electrochemical reducing power are adapted selectively to the reactor condition but other bacteria that can't generate biochemical reducing power from the electrochemical reducing power are not. The DNA bands were extracted from TGGE gel and sequenced. Identity of the bacteria was determined based on the 16S-rDNA sequence homology.

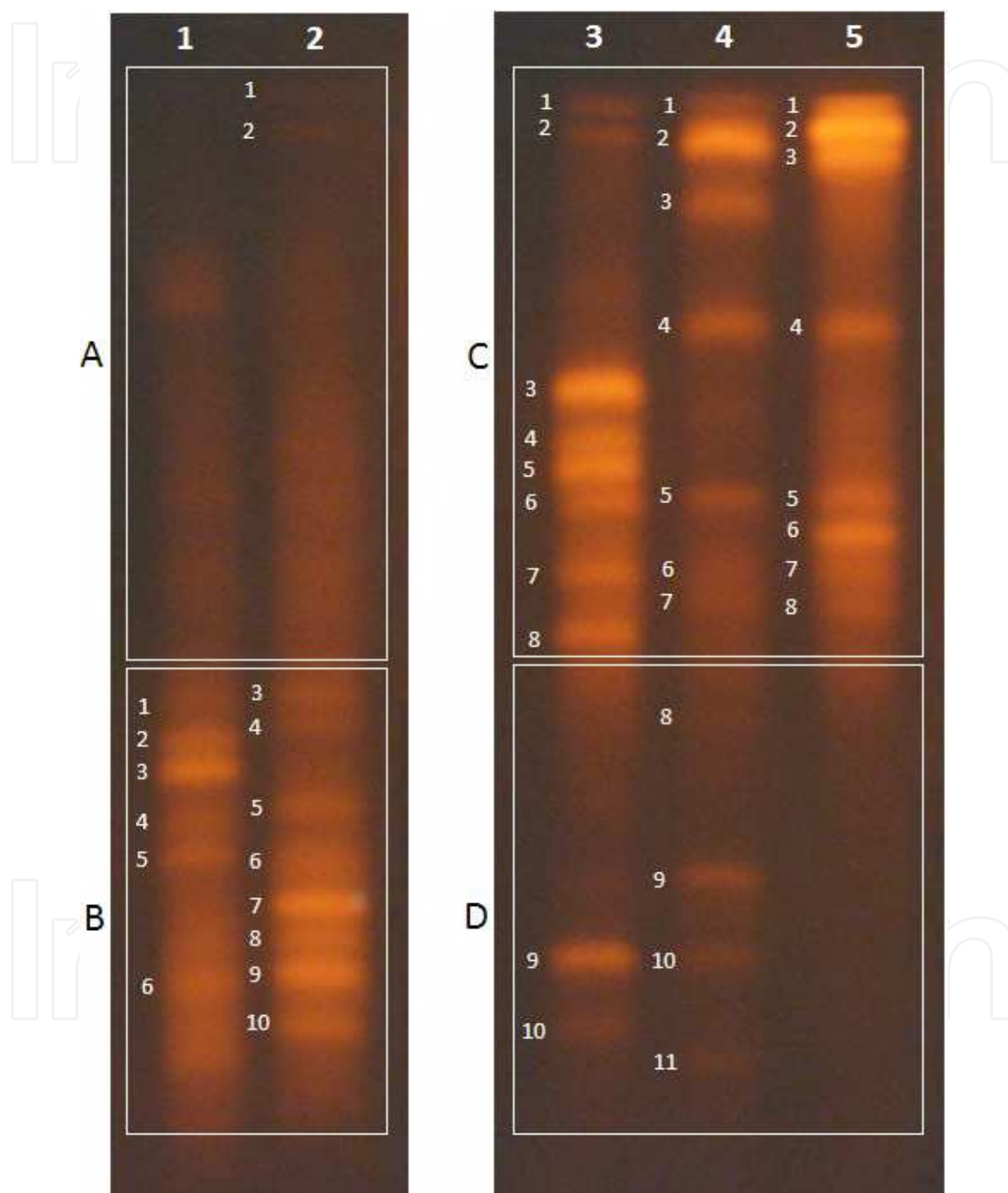


Fig. 10. TGGE patterns of 16S-rDNA variable regions amplified with chromosomal DNA extracted from bacterial communities enriched in the cylinder-type electrochemical bioreactors. 50 ml of bacterial culture was isolated from the electrochemical bioreactor at the initial time immediately after inoculation (lane1), 2<sup>nd</sup> week (lane 2), 8<sup>th</sup> week (lane 3), 16<sup>th</sup> week (lane 4), and 24<sup>th</sup> week of incubation time (lane 5).

Lane	Band	Genus or Species	Homology (%)	Accession No.
1 (initial)	1	Uncultured <i>Burkholderia</i> sp.	98	FJ393136
	2	Groundwater biofilm bacterium	98	FJ204452
	3	<i>Hydrogenophaga</i> sp.	98	FM998722
	4	Uncultured bacterium sp.	97	HM481230
	5	<i>Aquamicrobium</i> sp.	98	GQ254286
	6	Uncultured <i>Actinobacterium</i> sp.	99	FM253013
2 (2 <sup>nd</sup> week)	1	Uncultured bacterium sp.	97	AF234127
	2	Uncultured bacterium sp.	97	EU532796
	3	Uncultured <i>Clostridium</i> sp.	99	FJ930072
	4	Uncultured <i>Polaromonas</i> sp.	99	HM486175
	5	Uncultured <i>Rhizobium</i> sp.	100	FM877981
	6	<i>Raoultella planticola</i>	98	EF551363
	7	Unidentified bacterium	98	AV669107
	8	Uncultured bacterium	99	HM920740
	9	Uncultured bacterium	97	GQ158957
	10	Uncultured <i>Klebsiella</i> sp.	98	GQ416299
3 (8 <sup>th</sup> week)	1	Uncultured bacterium sp.	97	AF234127
	2	Uncultured bacterium sp.	97	EU532796
	3	<i>Enterococcus</i> sp.	98	DQ305313
	4	Uncultured bacterium	98	HM820223
	5	<i>Aerosphaera taera</i>	99	EF111256
	6	<i>Alcaligenes</i> sp.	98	GQ383898
	7	Uncultured bacterium	98	HM231340
	8	Uncultured bacterium sp.	97	FJ675330
	9	<i>Stenotrophomonas</i> sp.	98	EU635492
	10	Uncultured <i>Klebsiella</i> sp.	98	GQ416299
4 (16 <sup>th</sup> week)	1	Uncultured bacterium sp.	97	AF234127
	2	Uncultured bacterium sp.	97	EU532796
	3	Uncultured bacterium sp.	98	HM575088
	4	<i>Alcaligenes</i> sp.	98	GQ200556
	5	<i>Alcaligenes</i> sp.	98	GQ383898
	6	Uncultured bacterium	98	HM231340
	7	<i>Achromobacter</i> sp.	96	GQ214399
	8	Uncultured <i>Lactobacillales</i> bacterium sp.	96	HM231341
	9	Uncultured <i>Ochrombacterium</i> sp.	97	EU882419
	10	<i>Stenotrophomonas</i> sp.	98	EU635492
	11	<i>Tissierella</i> sp.	96	GQ461822
5 (24 <sup>th</sup> week)	1	Uncultured bacterium sp.	97	AF234127
	2	Uncultured bacterium sp.	97	EU532796
	3	Uncultured bacterium sp.	98	HM820116
	4	<i>Alcaligenes</i> sp.	98	GQ200556
	5	<i>Alcaligenes</i> sp.	97	GQ383898
	6	<i>Enterococcus</i> sp.	99	FJ513901
	7	Uncultured bacterium	98	HM231340
	8	<i>Achromobacter</i> sp.	96	GQ214399

Table 1. The homologous bacterial species with the sequences of DNA extracted from TGGE bands (Fig 10), which were identified based on the GenBank database.

Some anaerobic bacteria (*Hydrogenophaga* sp. and *Clostridium* sp.) that may be originated from the anaerobic wastewater treatment reactor are detected at the initial cultivation time but disappeared after 8<sup>th</sup> week of incubation time (Kang and Kim, 1999; Willems et al., 1989; Lamed et al., 1988). On the other hand, the bacteria that are capable of fixing carbon dioxide by autotrophic or mixotrophic metabolism were enriched as shown in Table 1. All of the enriched bacteria may not be the carbon dioxide-fixing bacteria but *Achromobacter* sp. and *Alcaligenes* sp. are known to fix carbon dioxide autotrophically or mixotrophically (Freter and Bowien, 1994; Friedrich, 1982; Hamilton et al., 1965; Leadbeater and Bowien, 1984; Ohmura et al.). During the enrichment of the carbon dioxide-fixing bacteria, carbon dioxide consumption was increased and reached to stationary phase after 15<sup>th</sup> week of incubation time as shown in Fig 11. Various organic compounds contained in the bacterial cultures that were originated from anaerobic wastewater treatment reactor, aerobic wastewater treatment reactor, and forest soil might be consumed completely and then carbon dioxide-fixing bacteria might grow selectively. The carbon dioxide consumption was increased initially and then reached to stationary phase after 15<sup>th</sup> week of incubation time, which is proportional to the enrichment time of the *Achromobacter* sp. and *Alcaligenes* sp.

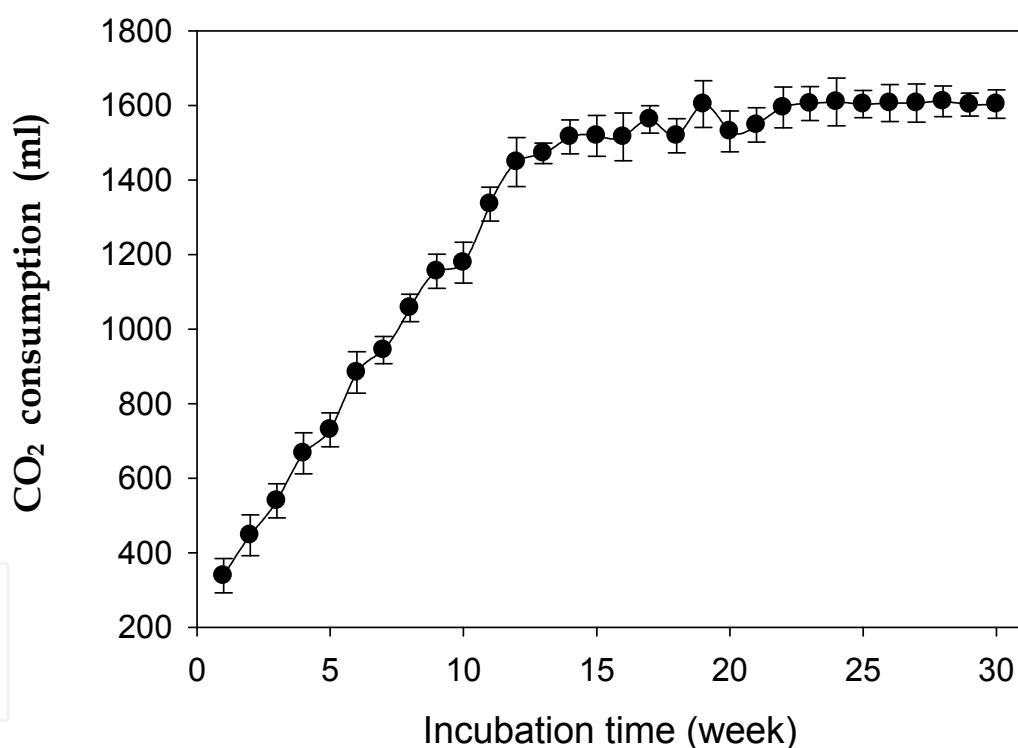


Fig. 11. Weekly consumption of CO<sub>2</sub> in the electrochemical bioreactor from the initial incubation time to 30 weeks. CO<sub>2</sub> consumption was analyzed weekly and the gas reservoir was refilled with 50±1% of CO<sub>2</sub> to N<sub>2</sub> at 4-week intervals.

Before and after enrichment, the bacterial community grown in the cylinder-type electrochemical bioreactor was analyzed using the pyrosequencing technique (Van der Bogert et al., 2011). The classifiable sequences obtained by the pyrosequencing were identified based on the Ribosomal Database Project (RDP), and defined at the 100 % sequence homologous level. The most abundant sequences (17.96%) obtained from the bacterial culture before enrichment was identified as *Brevundimonas* sp., and the abundance

of sequences identified with *Alcaligenes* sp. and *Achromobacter* sp. was 0.98 and 0.12%, respectively. Meanwhile, the most abundant sequences (43.83%) obtained from the bacterial culture after enrichment was identified as *Achromobacter* sp., and the most classifiable sequences were also identified as *Achromobacter* sp. and *Alcaligenes* sp. as shown in Table 2.

Before enrichment				After enrichment			
Classifiable sequences	Abundance (%)	Bacterial genus	Homology (%)	Classifiable sequences	Abundance (%)	Bacterial genus	Homology (%)
876	17.96	<i>Brevundimonas</i>	100	2248	43.83	<i>Achromobacter</i>	100
153	3.14	<i>Pseudomonas</i>	100	748	14.58	<i>Achromobacter</i>	100
111	2.28	<i>Hydrogenophaga</i>	100	595	5.87	<i>Stenotrophomonas</i>	100
99	2.03	<i>Delftia</i>	100	301	2.28	<i>Achromobacter</i>	100
86	1.76	<i>Stenotrophomonas</i>	100	263	1.77	<i>Achromobacter</i>	100
70	1.44	<i>Pseudomonas</i>	100	219	1.23	<i>Achromobacter</i>	100
53	1.09	<i>Parvibaculum</i>	100	117	0.90	<i>Achromobacter</i>	100
52	1.07	<i>Brevundimonas</i>	100	91	0.66	<i>Achromobacter</i>	100
48	0.98	<i>Alcaligenes</i>	100	63	0.57	<i>Alcaligenes</i>	100
32	0.66	<i>Comamonas</i>	100	46	0.53	<i>Achromobacter</i>	100
31	0.64	<i>Bacillus</i>	100	34	0.49	<i>Achromobacter</i>	100
26	0.53	<i>Bosea</i>	100	29	0.49	<i>Castellaniella</i>	100
21	0.43	<i>Devosia</i>	100	27	0.45	<i>Achromobacter</i>	100
17	0.35	<i>Acidovorax</i>	100	25	0.45	<i>Achromobacter</i>	100
12	0.25	<i>Brevundimonas</i>	100	25	0.39	<i>Stenotrophomonas</i>	100
12	0.25	<i>Sphaerobacter</i>	100	23	0.16	<i>Achromobacter</i>	100
11	0.23	<i>Brevundimonas</i>	100	23	0.14	<i>Alcaligenes</i>	100
9	0.18	<i>Acinetobacter</i>	100	20	0.12	<i>Achromobacter</i>	100
9	0.18	<i>Sphaerobacter</i>	100	14	0.10	<i>Alcaligenes</i>	100
8	0.16	<i>Brevundimonas</i>	100	14	0.10	<i>Pseudomonas</i>	100
7	0.14	<i>Hyphomicrobium</i>	100	11	0.08	<i>Achromobacter</i>	100
7	0.14	<i>Thermomonas</i>	100	10	0.08	<i>Achromobacter</i>	100
6	0.12	<i>Achromobacter</i>	100	8	0.06	<i>Achromobacter</i>	100
6	0.12	<i>Brevundimonas</i>	100	7	0.06	<i>Achromobacter</i>	100
4	0.10	<i>Devosia</i>	100	7	0.06	<i>Achromobacter</i>	100
3	0.08	<i>Pseudoxanthomonas</i>	100	6	0.04	<i>Alcaligenes</i>	100
3	0.06	<i>Castellaniella</i>	100	6	0.04	<i>Achromobacter</i>	100
3	0.06	<i>Gordonia</i>	100	6	0.04	<i>Achromobacter</i>	100

Table 2. Relative abundances of dominant bacterial taxa in the bacterial culture before and after enrichment. The relative abundances were estimated from the proportion of classifiable sequences, excluding those sequences that could not be classified below the genus level and 100% homology with the specific bacterial genus.



The *Achromobacter* sp. described in previous research was a facultative chemoautotroph (Hamilton *et al.*, 1965; Romanov *et al.*, 1977); however, it grew autotrophically with electrochemical reducing power under a CO<sub>2</sub> atmosphere and consumed CO<sub>2</sub> in this study. This result demonstrates that *Achromobacter* sp. grown in the electrochemical bioreactor may be a chemoautotroph capable of fixing CO<sub>2</sub> with the electrochemical reducing power. Meanwhile, various articles have reported that *Alcaligenes* sp. grew autotrophically (Frete and Bowien, 1994; Doyle and Arp, 1987; Leadbeater and Bowien, 1984) or heterotrophically (Reutz *et al.*, 1982). According to these articles, *Alcaligenes* spp. are capable of growing autotrophically with a gas mixture of H<sub>2</sub>, CO<sub>2</sub>, and O<sub>2</sub>, as well as heterotrophically under air on a broad variety of organic substrates. *Alcaligenes* spp. metabolically oxidize H<sub>2</sub> to regenerate the reducing power during autotrophic growth under H<sub>2</sub>-CO<sub>2</sub> atmosphere (Hogrefe *et al.*, 1984). The essential requirement for the autotrophic growth of both *Achromobacter* spp. and *Alcaligenes* spp. under CO<sub>2</sub> atmosphere is to regenerate reducing power in conjunction with metabolic H<sub>2</sub> oxidation, which may be replaced by the electrochemical reducing power on the basis of the results obtained in this research. The electrochemical reducing power required for the cultivation of carbon-dioxide fixing bacteria can be produced completely by the solar panel, by which atmospheric carbon dioxide may be fixed by same system to the photosynthesis.

## 6. Strategy of atmospheric carbon dioxide fixation using the solar energy

In global ecosystem, land plants, aquatic plants, and photoautotrophic microorganisms produce biomass that is original source of organic compounds (O'Leary, 1988). Autotrophs that are growing naturally or cultivating artificially have fixed the atmospheric carbon dioxide generated by heterotrophs, by which the atmospheric carbon dioxide may be balanced ecologically. However, the carbon dioxide generated from the combustion of organic compounds (petroleum and coal) that are not originated from biomass may be accumulated additionally in the atmosphere, inland water, and sea water. The solar radiation that reaches to the earth may not be limited for photosynthesis of phototrophs or electric generation of solar cells; however, the general habitats for growth of the phototrophs have been decreased by various human activities and the places for installation of the solar cells are limited to the habitats for human. If the solar cells were installed in the natural habitats, phototrophic fixation of carbon dioxide may be decreased in proportion to the electricity generation by the solar cells. The constructions of new cities, farmlands, golf courses, ski resorts, and sport grounds cause to convert the forests to grass field whose ability for carbon dioxide fixation is greatly lower than the forest. Consequently, the plantation of trees and grasses in the habitable lands or cultivation of algae and cyanobacteria in the habitable waters can't be the way to decrease additionally the atmospheric carbon dioxide.

Carbon dioxide has been fixed biologically by photoautotrophic, chemoautotrophic and mixotrophic organisms. The photoautotrophic bacteria assimilate carbon dioxide into organic compounds for cell structures with reducing power regenerated by the solar radiation under atmospheric condition (Kresge *et al.*, 2005). The chemoautotrophs assimilate carbon dioxide into cell structure in coupling with production of methane or acetic acid with reducing power regenerated by hydrogenase under strict anaerobic hydrogen atmosphere (Perreault *et al.*, 2007). The mixotrophs assimilate carbon dioxide into biomolecules with reducing power regenerated in coupling with metabolic oxidation of organic or inorganic compounds (Eiler, 2006). The photoautotrophs, chemoautotrophs, and mixotrophs can reduce metabolically carbon dioxide to organic carbon with the common reducing power (NADH or NADPH), which, however, are regenerated by

different metabolisms. The photoautotrophs, especially cyanobacteria that fix carbon dioxide by completely same metabolism (Calvin cycle) with plants, appear as if they are ideal organism to fix biologically carbon dioxide without chemical energy; however, they are unfavorable to be cultivated in the tank-type bioreactor owing to the limitation of reachable distance of solar radiation in aquatic condition. The chemoautotrophs may be useful to produce methane and acetic acid from carbon dioxide; however, they can grow only in the limit condition of the lower redox potential than  $-300$  mV (vs. NHE) and with hydrogen. The mixotrophs can grow in the condition with electron donors, which are regardless of organic or inorganic compounds, for regeneration of reducing power under aerobic and anaerobic condition. This is the reason why the facultative anaerobic mixotrophs may be more effective than others to fix the atmospheric carbon dioxide directly by simple process. Especially, the cylinder-type electrochemical bioreactor equipped with the built-in anode compartment (Fig 9) is an optimal system for the cultivation or enrichment of facultative anaerobic mixotrophs. Basements of buildings or villages are used generally for maintenances or facilities for wastewater collection, electricity distribution, tap water distribution, and garage. The basements can't be the habitats for cultivation of plants with the natural sun light but can be utilized for cultivation of the carbon dioxide-fixing bacteria with electric energy generated from the solar cells that can be installed on the rooftop as shown in Fig 12.

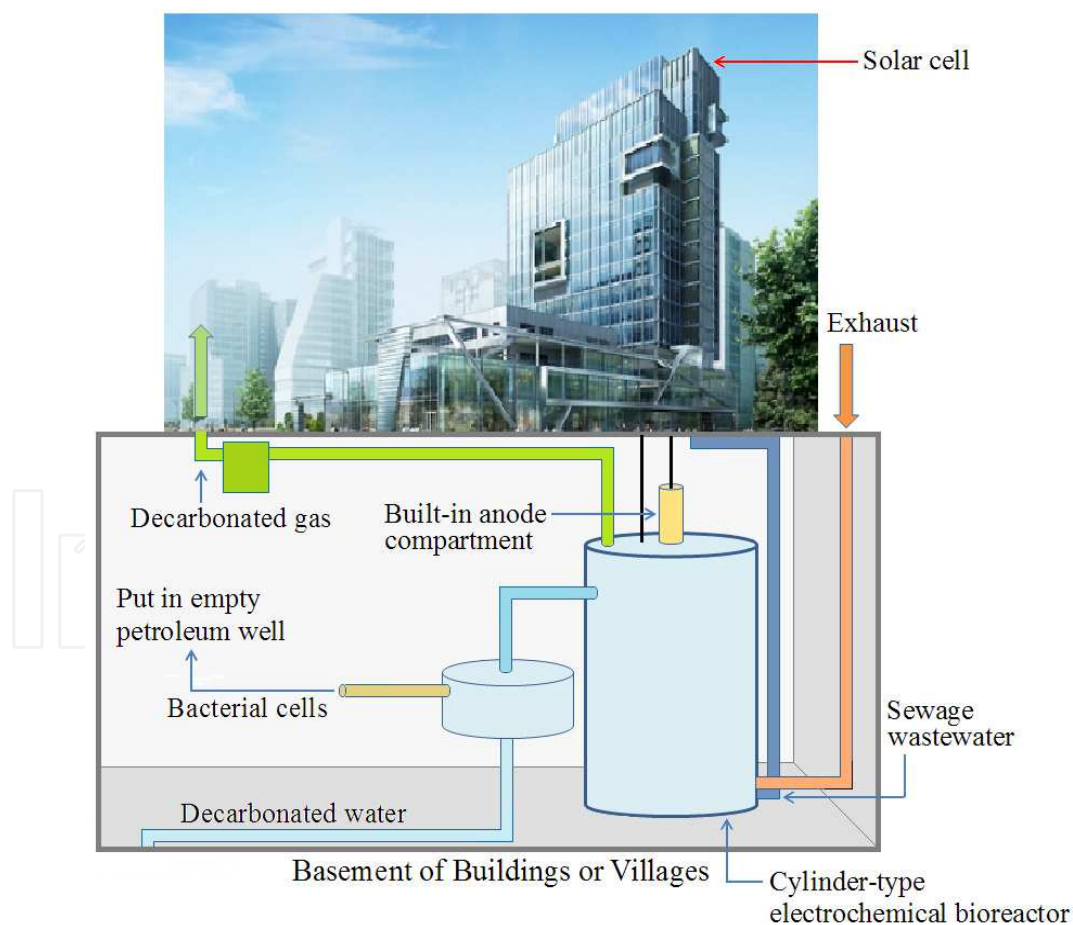


Fig. 12. Schematic structure of the electrochemical bioreactors installed in the building basement. The carbon dioxide-fixing bacteria can be cultivated using the electric energy generated by the solar cells.

The facultative anaerobic mixotrophs assimilate heterotrophically organic compounds contained in the wastewater into the structural compounds of bacterial cells under oxidation condition but autotrophically carbon dioxide into the biomass under condition with high balance of biochemical reducing power (NADH/NAD<sup>+</sup>). DC electricity generated from the solar cells can be transferred very conveniently to the cylinder-type electrochemical bioreactor without conversion, which is the energy source for increase of biochemical reducing power balance. A part of the atmospheric carbon dioxide has been generated from the combustion system of fossil fuel, which may be required to be return to the empty petroleum well. To store the bacterial cells in the empty petroleum well is to return the carbon dioxide generated from petroleum combustion to the original place. The peptidoglycans, phospholipids, proteins, and nucleic acids that are major ingredients of bacterial cell structures are stable chemically to be stored in the empty petroleum well owing to the non-oxygenic condition. Conclusively, what the atmospheric carbon dioxide originated from the petroleum and coal is returned to the original place again may be best way to decrease the greenhouse effect.

## 7. Conclusion

The atmospheric carbon dioxide originated from petroleum and coal is required to be completely isolated from the ecological material cycles. The carbons in the ecological system are accumulated as the organic compounds in the organisms and as the carbon dioxide in the atmosphere, which is cycled via the photosynthesis and respiration, especially, plants are the biggest pool for carbon storage. However, the forest and plant-habitable area has been decreased continuously by human activities.

The cultivation of cyanobacteria and single cell algae with solar energy may be the best way to isolated effectively carbon dioxide from atmosphere but is possible in the water pool-type reactor located in the plant-habitable area. In other words, the forests or grass lands may be replaced by the water pools, by which the effect of carbon dioxide fixation has to be decreased. The cyanobacteria and algae can be cultivated in the bioreactor using lamp light operated with electric energy that is generated from solar cells, for which the solar energy has to be converted to electric energy and then converted again to the light energy. These phototrophic microorganisms have been studied actively and applied to produce nutrient sources and pharmacy. The goal for cultivation of the phototrophic microorganisms is to produce the utilizable materials but not to fix carbon dioxide like the agricultural purpose.

The carbon compounds of the organic nutritional compounds contained in the sewage wastewater are the potential carbon dioxide, which may be the useful medium for cultivation of the mixotrophic bacteria capable of fixing carbon dioxide. The maximal balance of anabolism to catabolism is theoretically 0.4 to 0.6 in the mixotrophic bacteria growing with organic carbons as the energy source, in which the carbon dioxide can't be the source for both anabolism and catabolism; however, the balance can be changed by the external energy like the electrochemical reducing power. In the condition with both the organic carbons and the electrochemical reducing power as the energy source, the balance of anabolism to catabolism may be increased to be higher than 0.4 due to the carbon dioxide assimilation that is generated in coupling with the redox reaction of

biochemical reducing power electrochemically regenerated. The electrochemical reducing power can induce regeneration of NADH and ATP, by which both the assimilation of organic carbon and carbon dioxide into bacterial structure compounds can be activated. The goal of cultivation of bacterial cells using the cylinder-type electrochemical is to assimilate the atmospheric carbon dioxide to the organic compounds for bacterial structure without the combustion of fossil fuel and without production of metabolites. Some metabolites that are methane and acetic acid can be generated by the strict anaerobic bacteria under anaerobic hydrogen-carbon dioxide atmosphere but not useful for industrial utility owing to the cost for production. Meanwhile, the methane and acetic acid produced from the organic compounds in the process for treatment of wastewater or waste materials may be useful as the by-product for the industrial utility. The cell size and structural character of bacteria permits to put directly the bacterial cells in the empty petroleum well without any process, by which the atmospheric carbon dioxides are returned to the original place.

## 8. Acknowledgement

Writing of this chapter was supported by the New & Renewable Energy of the Korea Institute of Energy Technology Evaluation and Planning (KETEP) grant funded by the Korea government Ministry of Knowledge Economy (2010T1001100334)

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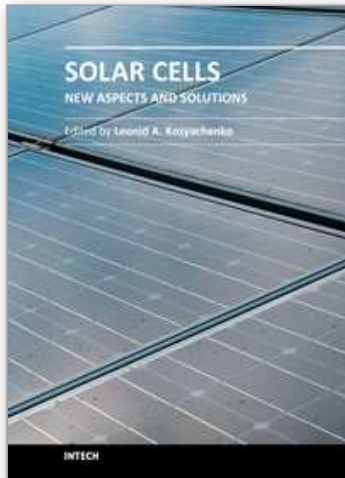
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## **Solar Cells - New Aspects and Solutions**

Edited by Prof. Leonid A. Kosyachenko

ISBN 978-953-307-761-1

Hard cover, 512 pages

**Publisher** InTech

**Published online** 02, November, 2011

**Published in print edition** November, 2011

The fourth book of the four-volume edition of 'Solar cells' consists chapters that are general in nature and not related specifically to the so-called photovoltaic generations, novel scientific ideas and technical solutions, which has not properly approved. General issues of the efficiency of solar cell and through hydrogen production in photoelectrochemical solar cell are discussed. Considerable attention is paid to the quantum-size effects in solar cells both in general and on specific examples of super-lattices, quantum dots, etc. New materials, such as cuprous oxide as an active material for solar cells, AlSb for use as an absorber layer in p-i-n junction solar cells, InGaAsN as a promising material for multi-junction tandem solar cells, InP in solar cells with MIS structures are discussed. Several chapters are devoted to the analysis of both status and perspective of organic photovoltaics such as polymer/fullerene solar cells, poly(p-phenylene-vinylene) derivatives, photovoltaic textiles, photovoltaic fibers, etc.

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Doo Hyun Park, Bo Young Jeon and Il Lae Jung (2011). Bioelectrochemical Fixation of Carbon Dioxide with Electric Energy Generated by Solar Cell, Solar Cells - New Aspects and Solutions, Prof. Leonid A. Kosyachenko (Ed.), ISBN: 978-953-307-761-1, InTech, Available from:  
<http://www.intechopen.com/books/solar-cells-new-aspects-and-solutions/bioelectrochemical-fixation-of-carbon-dioxide-with-electric-energy-generated-by-solar-cell>

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